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Delineation of the Marfan Phenotype Associated With Mutations in Exons 23–32 of the *FBN1* Gene

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Marfan syndrome is a dominantly inherited connective tissue disorder with a wide range of phenotypic severity. The condition is the result of mutations in *FBN1*, a large gene composed of 65 exons encoding the fibrillin-1 protein. While mutations causing classic manifestations of Marfan syndrome have been identified throughout the *FBN1* gene, the six previously characterized mutations resulting in the severe, perinatal lethal form of Marfan syndrome have clustered in exons 24–32 of the gene. We screened 8 patients with either neonatal Marfan syndrome or severe cardiovascular complications of Marfan syndrome for mutations in this region of the gene. Using intron-based exon-specific primers, we amplified exons 23–32 from genomic DNAs, screened these fragments by single-stranded conformational polymorphism analysis, and sequenced indicated exons. This analysis documented mutations in exons 25–27 of the *FBN1* gene in 6 of these patients. These results, taken together with previously published *FBN1* mutations in this region, further define the phenotype associated with mutations in exons 24–32 of the *FBN1* gene, information important for the development of possible diagnostic tests and genetic counseling. © 1996 Wiley-Liss, Inc.

KEY WORDS: Marfan syndrome, *FBN1*, fibrillin-1, neonatal Marfan syndrome

INTRODUCTION

Marfan syndrome is a pleiotropic disorder of arachnodactyly, dolichostenomelia, scoliosis, pectus deformities, lens subluxation and myopia, mitral valve abnormalities, and aortic dilatation and dissection that may lead to early death if untreated [Murdoch et al., 1972; Pyeritz and McKusick, 1979; Pyeritz, 1993; Finkbohner et al., 1995]. Marfan syndrome is an autosomal-dominant trait, but approximately 25% of cases are sporadic [Pyeritz, 1990].

There is a strikingly wide range of clinical severity, with the most severe form of the disorder termed neonatal Marfan syndrome. These children have severe cardiac valve regurgitation and dilation of the proximal aorta, which usually lead to heart failure and death in the first year of life [Phornphutkul et al., 1973; Geva et al., 1987; Buntinx et al., 1991]. Skeletal manifestations, such as arachnodactyly, dolichostenomelia, and pectus deformities, are typically present. Such infants may also have congenital flexion contractures, "crumpled" ears, loose redundant skin, and a characteristic "senile" facial appearance.

Marfan syndrome is the result of mutations in *FBN1*, which encodes a large glycoprotein called fibrillin-1 that is found in the extracellular matrix in the form of microfibrils [Sakai et al., 1986; Pereira et al., 1993]. Mutations that cause Marfan syndrome are spread throughout the gene, from exons 2–65 [Dietz et al., 1991, 1992a,b, 1993a,b; Kainulainen et al., 1992; Godfrey et al., 1993; Stahl-Hallengren et al., 1994; Tynan et al., 1993; Hewett et al., 1993, 1994; Piersall et al., Hayward et al., 1994; Karttunen et al., 1994; Nijbroek et al., 1995]. In contrast, mutations in patients with neonatal Marfan syndrome have clustered in exons 24–32 of *FBN1* [Kainulainen et al., 1994; Milewicz and Duvic, 1994; Wang et al., 1995]. To delineate further the phenotype associated with mutations in this region of the gene, we screened 8 patients with severe manifestations of Marfan syndrome for mutations in exons 23–32 of the gene. Two patients had neonatal Marfan syndrome. The other 6 had severe cardiovascular complications that either required or were predicted to re-

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quire surgical repair under the age of 6 years. Some of these patients also had congenital contractures, abnormal ears, and facial anomalies. We have identified *FBN1* mutations in 6 of these patients, 2 in exon 25, 3 in exon 26, and 1 in exon 27. These results, taken together with the phenotype of previously published mutations, further define the phenotype associated with mutations in this region.

MATERIALS AND METHODS

Clinical Report

Partial clinical data on patients P249, P250, and P251 were reported previously and coded as 89-093, 90-002, and 89-178, respectively [Milewicz et al., 1992]. Subsequent clinical data on P249 are not available. In addition, the results of fibrillin synthesis, secretion, and extracellular matrix incorporation by dermal fibroblasts explanted from these individuals have been reported.

Patient P092 was born to phenotypically normal parents and was noted at birth to have congenital contractures, scoliosis, and arachnodactyly. At age 3 months she was found to have mitral valve regurgitation and trace aortic valve incompetence, scoliosis, and dislocation of the lens of her right eye. She had mitral valve regurgitation and failure to thrive due to progressive heart failure, and underwent mitral valvuloplasty and annuloplasty at age 22 months. At that time she had frontal bossing, deep-set eyes, and large floppy ears (Fig. 1A). An aortogram done before surgery showed marked mitral regurgitation, dilation of the sinuses of Valsalva with normal ascending aorta, and trivial aortic regurgitation. She died postoperatively of congestive heart failure secondary to worsening cardiac contractility.

GD182 was born at term to a G3P2 mother. At birth he was thought to have Marfan syndrome because of an abnormal face and arachnodactyly. He had a flexion contracture of his right great toe. An echocardiogram showed marked aortic root dilation (aortic root diameter of 1.83 cm, 5.7 standard deviations (SD) above the mean for body surface area), and an enlarged pulmonary artery. A follow-up echocardiogram 1 month later showed a progressively enlarging aortic root, and he was treated with propranolol (6 mg every 6 hr). Severe myopia with iridodonesis was present. Subsequent echocardiograms showed progressive aortic root enlargement, and he underwent a Bentall procedure at age 18 months, when his aortic diameter was 43 mm (18 SD above the mean). Postoperative echocardiograms showed mitral valve prolapse and regurgitation. At age 2 years, he had marked joint laxity, pectus excavatum, and an abnormal facial appearance with frontal bossing, deep-set eyes, micrognathia, dolichocephaly, and large, flaccid ears (Fig. 1B).

P250 was hospitalized at age 4 months for repair of a dislocated hip. Detection of a heart murmur prompted an echocardiogram, which demonstrated mitral valve prolapse and regurgitation with aortic root dilation. At that time he was also noted to have bilateral lens dislocation, a pectus deformity, thoracolumbar scoliosis, dolichostenomelia, and arachnodactyly. He had an abnormal facial appearance, with slight prominence of the sagittal sutures without cranial asymmetry, mild frontal bossing, deep-set eyes, large, flaccid ears, and a

Fig. 1. Photographs of patients P092 (**A**) and GD182 (**B**) demonstrating frontal bossing, deep-set eyes, micrognathia, and large ears.

highly arched palate. At age 26 months he underwent aspiration of a right subluxed lens by anterior vitrectomy. At age 6 years, the patient had his mitral valve repaired, at which time the aortic root diameter was 164% of normal when corrected for body surface area.

P251 was born at term to a G2P1 mother. Immediately after birth he experienced respiratory distress and was intubated. An echocardiogram showed a bicuspid aortic valve with right ventricular hypertrophy suggestive of pulmonary hypertension, tricuspid regurgitation, and increased right atrial size. He had dolichostenomelia, arachnodactyly, pectus excavatum, kyphosis, and contracted and lax joints. The patient also had bilateral lens dislocation and an abnormal facial appearance, with redundant skin and retrognathia. His ears were apparently low-set and "crumpled" with a prominent antihelix, and he had a highly arched palate. He died at age 2 months of cardiac failure.

Patient GD269 was born at 37 weeks of gestation to phenotypically normal parents. Two prenatal ultrasound examinations were reportedly normal. Immediately after birth she was noted to have multiple flexion contractures involving the wrists and elbows, limited extension of the hips and knees, and arachnodactyly. She also had an abnormal facial appearance and downturned superior helices of her ears. An echocardiogram demonstrated regurgitation of all four valves. Despite vigorous treatment, she died a week later. At autopsy, all four cardiac valves were nodular and redundant, and there was a dilated ascending aorta.

P252 was a 1-year-old girl who underwent evaluation for marked joint laxity, pectus excavatum, a markedly dilated aorta (19 mm, >95th confidence interval for body surface area [Sheil et al., 1995]) and pulmonary artery, and mitral valve prolapse. Her facial appearance was normal and she did not have lens dislocation. At age 2 years her aortic root was 32 mm (>95th confidence interval for body surface area).

P002 was born 6 weeks prematurely to a G3P2 mother. The patient was given the diagnosis of arthrogryposis at birth. She underwent bilateral Achilles tendon release operations at age 1 year and left hand tendon releases at age 3 years. She had marked lower limb joint laxity, requiring leg braces. At age 6 years her ascending aorta dissected and she underwent a Bentall procedure. At that time she had arachnodactyly, dolichostenomelia, pectus carinatum, and scoliosis. She did not have ectopia lentis.

Genomic DNA Extraction

Blood samples and skin biopsies were obtained from patients and parents after appropriate consent was signed. Fibroblasts were explanted from skin biopsies using previously described techniques [Milewicz et al., 1992]. Genomic DNA was obtained from white blood cells or dermal fibroblasts using standard techniques [Sambrook et al., 1989]. DNA from autopsy specimens was extracted according to previously published procedures [Hecht et al., 1993].

Genomic DNA Amplification

Exons 23–32 of the *FBN1* gene were amplified individually from genomic DNA, using primers generously provided by the National Marfan Foundation and Dr. Harry C. Dietz (Table I). Reactions were carried out under the following conditions: 50 ng of genomic DNA were amplified in a 25-μl reaction volume using 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 10 mM of each primer, and 0.5 U Taq polymerase. Polymerase chain reaction (PCR) conditions consisted of an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturing at 94°C for 30 sec, annealing at 58°C for 30 sec (except for exon 27, where 54°C was used), and extension at 72°C for 30 sec, with a final extension at 72°C for 10 min using a Perkin-Elmer Corporation thermal cycler and reagents (Norwalk, CT).

Single-Stranded Conformational Polymorphism (SSCP) Analysis

SSCP analysis was performed using amplified DNA fragments [Orita et al., 1989]. After denaturation for 2 min at 100°C in a solution of 95% deionized formamide and 10 mM NaOH, the DNA fragments were analyzed by electrophoresis through a nondenaturing 0.5× MDE gel (AT Biochem, Malvern, PA) at 4°C at 40 watts for 4 hr. The DNA fragments were then visualized using silver staining (Pierce, Rockford, IL).

DNA Sequencing

The amplified DNA fragments used for direct sequencing were purified using the Wizard PCR Prep DNA Purification System (Promega Corp., Madison, WI). DNA fragments with point mutations were sequenced using both the sense and antisense amplification primers to confirm the mutation. For exon 25, amplified from P251 genomic DNA, the DNA fragments were cloned into the pGEM-T cloning vector (Promega), and plasmids containing the inserted DNA were purified using the Wizard Miniprep DNA Purification System (Promega). Purified DNA fragments or plasmids

TABLE I. Primer Sequences Used to Amplify Exons 23–32 of *FBN1* Gene

Exon	Primer sequences	BP
23S	GTTTTATGAACTTACCAGGTTC	333
23AS	ACCGAAGCTAAGTGCTCAG	
24S	CAGCAAATTATTATGTGTGCAG	418
24AS	ATCAAGTAGAGTGCTGAGATC	
25S	CAAGAACTTCCAACCTTCATG	273
25AS	TTAAAGGACGTCCCCTCTC	
26S	AATTAAGGCTGTCCTGAGAC	227
26AS	CATGGAATCCTTCTCTTTCTG	
27S	GGCCCCCACCTTTAACATG	181
27AS	GAAAGTCTTTGCTCCTTAC	
28S	TGCCAAAGTTGGAAGCTTATG	225
28AS	TAACATAACATAACATAAAATAAAG	
29S	CAGACATCCAAACCATATCAG	213
29AS	GAACCTACTGAGAGATTCAAC	
30S	AATAGTCTTATGCTAGTAGGC	292
30AS	ACAGTGCTTATGACTAACAAG	
31S	GTACTCAATGATATCAAATAGC	230
31AS	ACCAATCTCTTAACTACTTAATA	
32S	CCAAAAGACATTTGTGCTGAG	226
32AS	GTGTAATCTATGCAGTCCTTG	

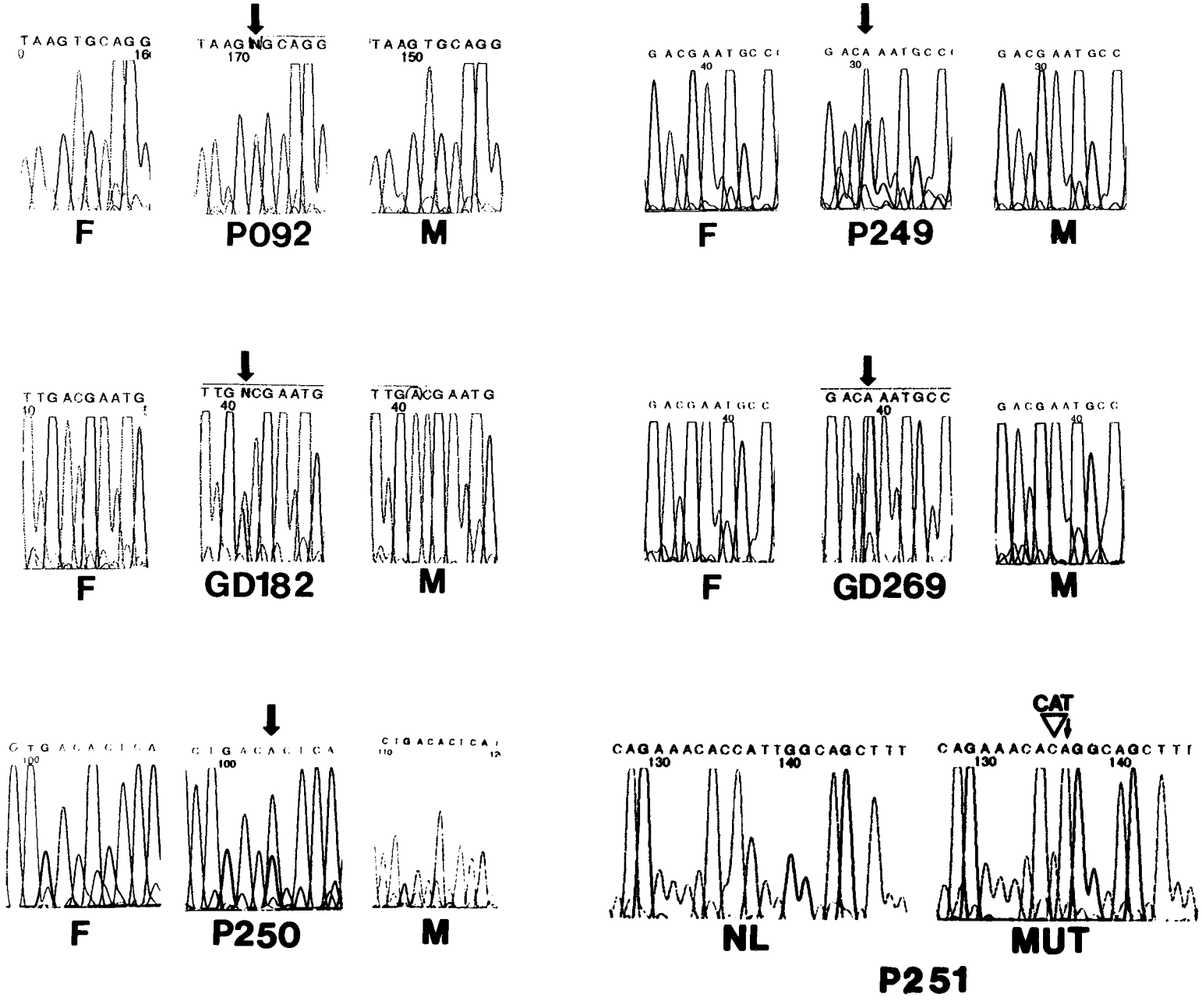

Fig. 2

were sequenced using an ABI automated sequencer model 373A (Applied Biosystems, Inc., Foster City, CA).

RESULTS

SSCP analysis and subsequent sequence analysis in both sense and antisense directions of the individually amplified exons from patients' DNA showed five missense mutations (Fig. 2). Patient P092 had a T to C transition at base 3157 in exon 25, causing substitution of an arginine for a cysteine at amino acid 1053 (nucleotide and amino acid sequences are numbered according to Pereira et al. [1993]). Amplification and sequence analysis of exon 25 using DNA from both parents were normal. Patient GD182 had an A to G transition at base 3215 in exon 26, resulting in substitution of glycine for aspartic acid at amino acid residue 1072. Sequence analysis of exon 26 of the parents' DNA determined that the identified transition was present in the child only. Patient P250 had a T to C transition at base 3349 in exon 27, which substituted glycine for cysteine at amino acid residue 1117. Again, sequence analysis of parental DNA showed that this was a new mutation in the child.

DNA from both P249 and GD269 contained the same missense mutation. The SSCP analysis of exon 26 amplified from both of these patients' DNA was abnormal (P249, Fig. 3B, lane 4). Sequence analysis of these DNAs showed a G to A transition at base 3217, resulting in substitution of lysine for glutamic acid at position 1073. The parents of both of these patients had wild-type sequence at this position.

SSCP analysis of DNA amplified from exon 25 of P251 resulted in a number of aberrantly migrating bands (Fig. 3A, lane 5). Exon 25 DNA was amplified and cloned, and five of these clones were sequenced. Three of the clones contained a deletion of bases 3141–3143, removing the codon for the isoleucine at amino acid 1048. In addition, in the same clones there was a T to A transversion at base 3144, which did not alter the threonine encoded at that site. The other two clones had normal sequence. SSCP analysis of 40 chromosomes from unrelated individuals indicated that this SSCP pattern was unique to P251.

Analysis of genomic DNA in this region from P002 and P252 failed to show causative *FBN1* mutations.

DISCUSSION

We have identified mutations in exons 25–27 of the *FBN1* gene in 6 patients with severe Marfan syndrome.

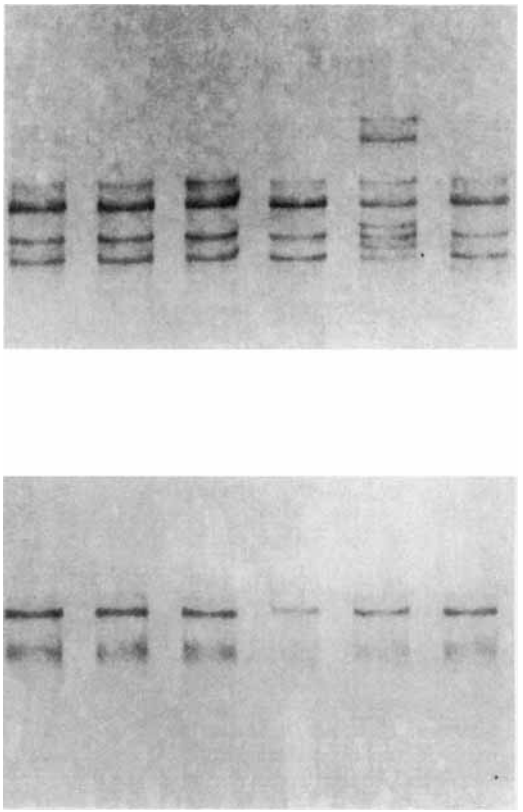

Fig. 3. SSCP analysis of DNA amplified from exon 25 (**A**) and exon 26 (**B**). Order of the samples is the same in both A and B. **Lanes 1 and 6** are amplified control DNA; **lane 2,** P252 DNA; **lane 3,** P250 DNA; **lane 4,** P249 DNA; **lane 5,** P251 DNA. Alterations observed in A, lane 5, and B, lane 4, are due to mutations.

Two of these patients (P251 and GD269) had manifestations of neonatal Marfan syndrome, including lethal cardiovascular complications, congenital contractures, "crumpled" helices of the ears, and typical face characterized by micrognathia, loose skin, and a "senile" appearance [Buntinx et al., 1991]. Four other patients had severe Marfan syndrome but did not have some of the manifestations associated with neonatal Marfan syndrome (P092, GD182, P250, and P249). All of these patients had surgically correctable cardiac complications. Three of these patients had an abnormal facial appearance that differed from that of infants with neonatal Marfan syndrome, with frontal bossing, deep-set eyes, and micrognathia (Fig. 1). The ears in these patients were large and flaccid, without crumpled helices. Flexion contractures were not present in 3 of these patients. These observations indicate that mutations in this region of the *FBN1* gene are associated not only with neonatal Marfan syndrome, but also with a severe form of Marfan syndrome that is characterized by abnormal face and ears, with or without congenital contractures.

FBN1 mutations in exons 24–32 that maintained the reading frame of the fibrillin-1 protein are summarized in Table II, along with the clinical findings of these patients. Examination of the phenotype associated with mutations in this region leads to several observations.

Fig. 2. Sequence analysis of PCR-amplified DNA fragments and cloned DNA. Individual exons of the FBN1 gene were amplified from genomic DNA from patients P092, GD182, P250, P249, and GD269, and sequenced directly using the sense (P092, GD182, P249, and GD269) or antisense primer (P250). The following heterozygous missense mutations were identified: P092, T3157C; GD182, A3215G; P250, T3349C; P249 and GD269, G3217A (arrows). Also shown is wild-type DNA sequence obtained from sequencing the same amplified exon from the respective patient's father's (F) or mother's (M) genomic DNA. Exon 25 amplified from genomic DNA from P251 was cloned, and five clones were sequenced, two of which showed normal or wild-type sequence (NL), and three of which showed mutant sequence (MUT). Mutated sequence contained a deletion of bases 3141–3143 (triangle) and a T to A transversion at base 3144 (small arrow).

TABLE II. *FBN1* Mutations in Exons 24–32 and Resulting Phenotype*

Mutation	Exon	Cardiovascular[a]	Congenital contractures	Abnormal face	Abnormal ears	Ectopia lentis	Reference/patient no.
K1023N	24	d, 20 hr	+	+	+	−	Kainulainen et al., 1994; Buntinx et al., 1991
Del I 1048	25	d, 2 months	+	+	+	+	P251
C1053R	25	d, 2 years	+	+	+		P092
1058 insC	25	d, 17 months	+	+	+	+	Milewicz and Duvic, 1994
Del exon 25	25	d, 12 hr	−			−	Kainulainen et al., 1994
D1072G	26	AV/AR s, 18 months	+	+	+	+	GD182
E1073K	26	MVP, ARD 1 year				+	P249
E1073K	26	d, 1 week	+	+	+		GD269
C1074R	26	d, 9 weeks	+	+	+	−	Kainulainen et al., 1994
C1117Y	27	AV/AR s, 27 years MV s, 28 years	−			+	Tynan et al., 1993
G1127S	27	MVP, AV/AR s, 72 years				−	Francke et al., 1995
C1117G	27	MV s, 6 years, MVR	−	+	+	+	P250
R1137P	27	Severe ARD, 19 years				+	Dietz et al., 1991
R1137P	27	AV/AR s, MV s, d, 17 years				+	Dietz et al., 1991
C1223Y	29	−				+	Hewett et al., 1994
C1242Y	30	MVP, AV/AR s, 19 years				+	Kainulainen et al., 1994
C1249S	30	ARD, 17 years	−			+	Dietz et al., 1992
Del exon 32	32	d, 3 months	+	+	+		Wang et al., 1995
Del exon 32	32	d, 5 months	+	+	+	+	Wang et al., 1995

*+, feature was present; −, feature not present; blank space, feature not reported, or patient not examined. All patients had arachnodactyly and dolichostenomelia, and many had pectus deformities. All mutations are sporadic.
[a]All patients died of complications related to their cardiovascular manifestations; all known causes of death were due to congestive heart failure. d, died; s, surgery; AV/AR, aortic valve/aortic root repair; ARD, aortic root dilation; MV, mitral valve repair; MVR, mitral valve regurgitation; MVP, mitral valve prolapse.

First, missense mutations in exons 24–26 and splicing errors deleting exon 32 result in lethal Marfan syndrome. These have been the only identified *FBN1* mutations described in patients with neonatal Marfan syndrome, with the exception of a neonatal case due to compound heterozygosity for *FBN1* mutations [Karttunen et al., 1994]. In addition, the 2 patients with neonatal Marfan syndrome whom we screened had mutations in exons 25 and 26. Although these numbers remain small, these findings indicate that this presentation is associated with mutations in this region. Second, all published reports of sporadic cases with contractures and severe cardiovascular disease have mutations in the region encompassing exons 24–32 of the *FBN1* gene. A mutation was not found in patient P002, who had severe cardiovascular disease and congenital contractures, indicating that either the causative mutation may be located outside this restricted domain, or that the patient may have a different disorder. Finally, mutations in this region can also be associated with more classic manifestations of Marfan syndrome. There have been 2 patients with mutations in this region described as having classic Marfan syndrome, both of whom have mutations that alter cysteines, C1117Y [Tynan et al., 1993], and C1249S [Dietz et al., 1992b].

Two other patients with reported mutations in this region of *FBN1* display atypical phenotypes of Marfan syndrome. The first patient was diagnosed as having Marfan syndrome after the birth of a severely affected daughter. At birth, this child had ectopia lentis and skeletal findings of Marfan syndrome. She died of congenital heart disease at age 15 months. The mother had ectopia lentis and skeletal findings of Marfan syndrome, but no cardiovascular involvement at age 66 years. Presumably, the *FBN1* mutation (C1223Y) identified in the mother was transmitted to her daughter, whose *FBN1* mutation status was unknown [Hewett et al., 1994]. The second patient with an identified *FBN1* mutation in this region without typical Marfan syndrome belongs to a family with inherited thoracic aortic aneurysms with mild ocular and skeletal features [Francke et al., 1995]. The *FBN1* mutation at residue 1127 substitutes a serine for a glycine, which may not disrupt the epidermal growth factor (EGF)-like domain as severely as either cysteine substitutions or alterations that disrupt amino acids important for calcium binding.

We identified a recurrent *FBN1* mutation, the G to A transition (E1073K) in exon 26 found in P249 and GD269. Both patients had severe cardiovascular involvement, but P249 lived beyond the perinatal period, while GD269 died at age 1 week. This phenotypic variability in the severity of disease caused by the same mutation is also seen in familial cases of Marfan syndrome [Pyeritz and McKusick, 1979]. Other factors in these patients may be responsible for this variability.

Coincidentally, the first identified *FBN1* mutation also occurred in this region of the gene, a G to a C transversion at nucleotide 3410 in exon 27 that was found in 2 patients with classic Marfan syndrome [Dietz et al., 1991]. The substitution of proline for arginine at this site in an EGF-like domain may be the reason for the less severe phenotype. Another mutation identified here alters the same codon as a previously published mutation in an unrelated Marfan patient. In P250, nucleotide 3349 is altered, changing cysteine 1117 to a glycine. A patient characterized as having classic Marfan syndrome had nucleotide 3350 altered, changing the same cysteine to a tyrosine [Tynan et al., 1993]. The varied phenotype associated with alterations in the same codon may reflect which amino acid is substituted for the cysteine or, alternatively, other unidentified factors.

The severe phenotype associated with these specific mutations in this region of the gene suggests a critical function for these domains of the fibrillin-1 protein. Fibrillin-1 is a large, cysteine-rich glycoprotein (350 kD) that has a repeated domain structure (Fig. 4A). There are 47 epidermal growth factor precursor (EGF)-like domains containing six cysteines each, and in 43 of 47 of these domains a calcium-binding consensus sequence is present [Corson et al., 1993; Pereira et al., 1993]. Seven domains with eight cysteines each are similar to a domain found in transforming growth factor β1-binding protein (TGF-β1-BP). There are two copies of an eight-cysteine hybrid motif which appear to contain both EGF and TGF-β1-BP-like sequences. In addition, there are three unique domains [Pereira et al., 1993].

The domains encoded by exons 25–36 are found midway through the protein and constitute the longest stretch of EGF-like domains in the protein (Fig. 4A). Exon 24 encodes an eight-cysteine domain found immediately amino-terminal to this stretch of EGF-like domains. The relative location of the EGF-like domains and the eight-cysteine domain are conserved in this region between fibrillin-1 and TGF-β1-BP (Fig. 4B) [Kanzaki et al., 1990]. TGF-β1-BP is a protein that is found associated with TGF-β1 in the extracellular space. TGF-β1-BP plays a role in the assembly and secretion of TGF-β1, and is thought to target TGF-β1 to particular extracellular matrix sites [Taipale et al., 1994]. TGF-β1 binds to cell surface receptors, where it is thought to have an important role in controlling the production and structure of the extracellular matrix, along with affecting cell growth, morphology, and differentiation [Kingsley, 1994]. The homology of fibrillin-1 and TGF-β1-BP raises the possibility that fibrillin-1 binds TGF-β1 during development. Mutations that result in the severe Marfan phenotype map into this TGF-β1-BP/fibrillin homology region, which raises the possibility that disruption of the extracellular targeting of the action of TGF-β1 during development explains the more severe phenotype.

The interaction of fibrillin-1 with TGF-β1 could also explain why some fibroblast cell strains from patients with neonatal Marfan syndrome have decreased production of decorin, a small chondroitin-dermatan sulfate proteoglycan found in the extracellular matrix [Pulkkinen et al., 1990; Raghunath et al., 1993; Superti-Furga et al., 1992]. Decorin binds TGF-β1, and neutralizes the activity of the growth factor [Yamaguchi et al., 1990]. In turn, the synthesis of decorin by various cell types is stimulated by TGF-β1

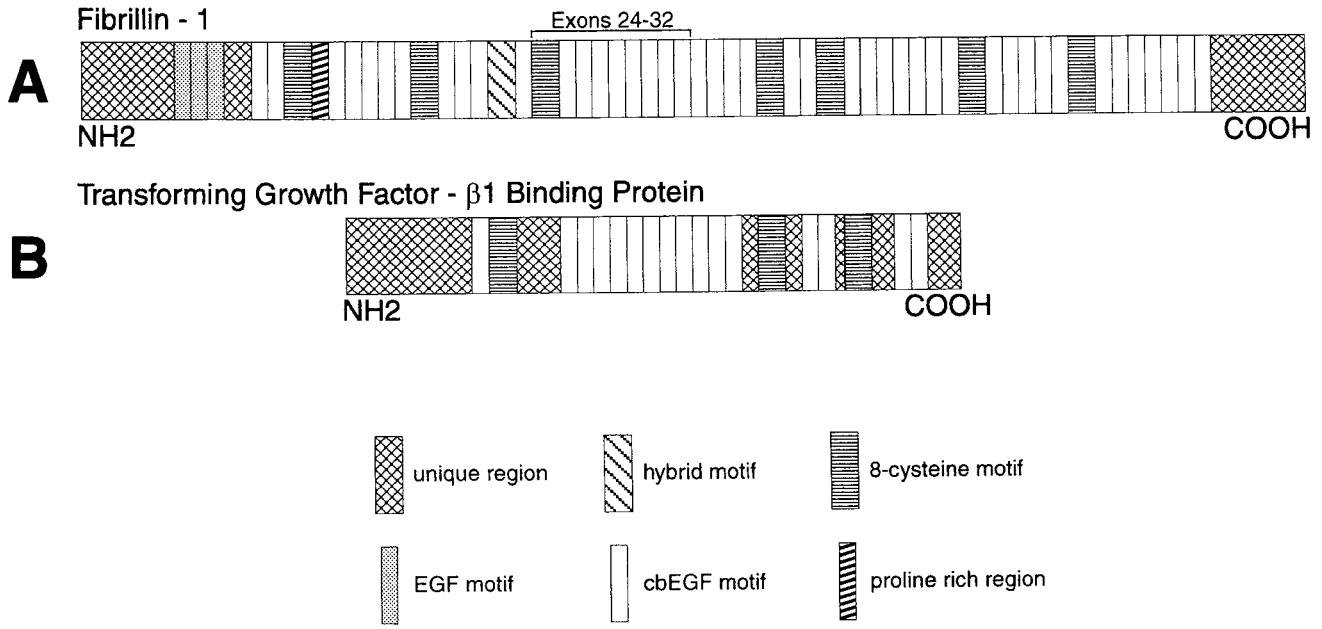

Fig. 4. Diagrammatic representation of protein domains in the fibrillin-1 (**A**) and TGF-β1 (**B**) binding protein. Different domains of these proteins are labeled in figure. Domains of fibrillin encoded by exons 24–38 are shown on the schematic of fibrillin-1 protein.

[Bassols and Massague, 1988]. This evidence indicates that decorin is an effector molecule in a negative feedback loop that regulates TGF-β1 activity [Ruoslahti and Yamaguchi, 1991]. The altered expression of decorin by dermal fibroblasts from patients with neonatal Marfan syndrome may reflect abnormal regulation of TGF-β1 due to altered interaction of TGF-β1 with mutant fibrillin-1 proteins.

Alternatively, mutations in this region of fibrillin-1 may be more disruptive to microfibril formation. Fibrillin-1 forms multimers that are microscopically visible as microfibrils. Microfibrils made by dermal fibroblasts have a characteristic structure when examined by rotary shadowing electron microscopy, consisting of beaded domains with a periodicity of 50–55 nm and interbeaded domains [Kielty et al., 1994]. Although mutations throughout the *FBN1* gene have been shown to disrupt fibrillin-1 incorporation into microfibrils, exons 24–32 may encode a region of fibrillin-1 with a unique function in the multimerization of the protein into stable microfibrils. Immunostaining of fibroblast cultures from neonatal Marfan syndrome patients shows not only an apparent decrease in fibrillin accumulation, but also an abnormal microfibril morphology [Superti-Furga et al., 1992; Godfrey et al., 1995]. In contrast to microfibrils formed by classic Marfan syndrome fibroblasts, the fibrils formed by neonatal Marfan syndrome fibroblasts are short, fragmented, and frayed. Therefore, alterations in this region of the protein have a significant and specific effect on microfibril formation, implying a unique role of this region in microfibril formation.

The cellular metabolism of fibrillin has been studied using explanted dermal fibroblasts in 3 of the patients reported here, P249, P250, and P251 [Milewicz et al., 1992]. Cells from P250 and P251 showed delayed secretion of fibrillin from the cells. The mutation in P250 alters the same cysteine (C1117) as a previously reported mutation in an unrelated Marfan patient [Tynan et al., 1993], and fibrillin studies on cells from that patient also showed delayed secretion [Aoyama et al., 1993]. All mutations leading to delayed secretion of fibrillin have disrupted or inserted a cysteine in one of the EGF-like domains, leading to the conclusion that correct disulfide bonding within EGF-like domains is necessary for proper secondary structure of the protein [Aoyama et al., 1993]. Although the mutation in P251 does not replace a cysteine, it deletes an amino acid from a loop of the EGF-like domain that is highly conserved in length, and so may interfere with correct folding [Maslen et al., 1991]. The mutation in P249 (E1073K) replaces an amino acid (glutamic acid) critical for calcium binding in an EGF-like domain [Cooke et al., 1987]; like disulfide bonding, calcium chelation is thought to be important for conformational stability. These cells (from P249) had normal fibrillin synthesis and secretion, but the secreted fibrillin was not incorporated into the pericellular matrix. The effect of these missense mutations on the secondary structure of EGF-like domains in this severe phenotype is similar to that of missense mutations elsewhere in the protein associated with classic Marfan syndrome. This implies that the location of a mutation, in addition to the type of mutation, is critical in producing the severe phenotype.

Defining the phenotype associated with mutations in exons 24–32 of *FBN1* has several clinical implications. First, screening of this region of *FBN1* could be used to confirm the diagnosis of neonatal Marfan syndrome in patients. Since a discrete region of the gene needs to be analyzed, this would be feasible and affordable with currently available techniques. Not only could this be used to confirm the diagnosis, but identification of a sporadic mutation in a neonatal Marfan patient is important for genetic counseling, since perinatal lethal Marfan syndrome can also result from compound heterozygous *FBN1* mutations [Karttunen et al., 1994]. In addition to patients with features of neonatal Marfan syndrome, patients with severe cardiovascular complications, with abnormal face and ears, may also be diagnosed by screening this region of *FBN1*. Future studies will determine the clinical utility of mutational *FBN1* analysis of exons 24–32 for diagnosis and genetic counseling.

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